WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Polymer Effects on Virus and Bacteria Transport in Subsurface

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Focus Categries: WQL, AG, GW

Congressional District: 1st

Descriptors: microorganisms, polyacrylamides, recharge, infiltration, ground water, vadose zone, virus, bacteria.

Critical Regional Water Related Problems

Water management strategies are closely related to management of water resources and water pollution. A water pollution problem which affects all areas with significant rainfall is soil erosion and the subsequent transport of soil and all land-based pollutants (pesticides, nutrients, pathogens, toxins etc.) which enter receiving waters such as coastal waters, estuaries, harbors, streams, rivers, and lakes. This problem is especially relevant to islands, coastal states, and other tropical regions which receive heavy rainfall or have a significant acreage under irrigation. A recent management strategy which has alleviated this problem is the application of anionic high molecular weight polymers such as polyacrylamides to soil as means to retain the structure of the soil and to prevent soil erosion and subsequent environmental problems.

This procedure is relatively new (< 7 years) but is now applied to approximately 750,000 acres in the US. This process has been used in furrow irrigated land and at construction sites for temporary erosion control but has potential for use in many other areas such as sprinkler irrigation, sediment detention basins, hillsides, and open land. Application of polymers has clearly shown a reduction in soil loss (95 percent or higher) and a 15-50% increase of water infiltration at these treated sites. Increase of water infiltration has several positive effects such as maintaining soil moisture, soil productivity, and in increasing the groundwater recharge. What is not clear is the effects of the use of polymers on the quality of groundwater if this process allows more of the water and its constituents to flow through the soil and finally to aquifers. Studies must be conducted immediately to evaluate if the use of polymers will allow water pollution constituents such as chemicals, nutrients, pesticides, and microbial pathogens to reach groundwater in aquifer. This question becomes more relevant today because of the increased level of water pollution by animal waste as well as the practice of reusing wastewater for irrigation and the recharge of groundwater supplies. In this regard, a major constituent of wastewater is fecal microorganisms, especially human enteric viruses that are most likely to be transported through the soil matrix to contaminate groundwater supplies and to cause water-borne diseases in humans.

This study was proposed to be conducted in two phases. During phase 1 (i.e., year 1) of this study, collection of basic data on the application method, dose, and effectiveness of polymers to stabilize different types of soils are being investigated. We are now investigating the ability of this polymer treated soil to enhance the penetration of chemicals (bromide and other reactive chemicals) and fluorescent microspheres through leaching experiments on soil columns. Preliminary results from year 1 show that the polymer treatment has enhanced the rate of water infiltration in some key soils of Hawaii. The results of the mobility of bromide and other reactive chemicals will be available in the next two months. The fluorescent microspheres proposed for use in this study have the surface characteristics of bacteria as well as bacterial viruses and they have been ordered from the vendors. The full results of phase 1 study will be available towards the end of the summer of 2000. The phase 2 or year 2 study will focus on the transport of two different types of fecal bacteria (gram negative rod, gram positive cocci) as well as bacterial viruses which have the same size, shape, and genetic constituents as human pathogen sewage borne viruses. We will also determine bacterial virus soil sorption and survival characteristics during phase 2 of this study and will enhance a model for the transport of these pathogens with and without the treatment of these polymers.

Results, Benefits, and/or other Information Expected

This study will evaluate the new land management practice of applying polymers to soil to prevent soil erosion by assessing its effect on enhancing the transport of soil pollutants (chemicals, nutrients, bacteria, and viruses) through polymer treated soils and determine its impact on ground-water quality. Carefully controlled laboratory studies will indicate if the chemicals and microspheres are moving deeper into soil profiles due to increased water flow and a possible reduction in sorption for reactive chemicals. Mathematical modeling, using available information for flow dynamics, sorption, and dispersion will indicate if the potential for transport of these chemicals and possibly pathogenic organisms to ground water will be higher with polymer amended soils compared to situations where no polymers are added.

Nature and Scope of Proposed Research

Agriculture is perceived to be a major non-point source polluter of surface and ground-water supplies. Both chemical (pesticides and nitrate) and fecal (bacteria, protozoa, and virus) pollution have been associated with agricultural land use practices. The proposed research addresses the potential problems (or benefits) associated with the use of high molecular weight polymers (anionic polyacrylamides, PAMs) on soils that have been treated with pathogenic wastes such as wastewater, biosolids (sludge), and manure. Some of the benefits of this research include the demonstration of the impact of soil amendments such as polymers and surface mulching on the transport of chemicals, fluorescent microspheres and spores during the first year and a more detailed study on the transport of bacteria, virus, and other organisms will be conducted during the second year. This will lead to the development of better management practices to reduce surface and ground water pollution.

Animal wastes (manure) and composts have traditionally been used for agricultural purposes throughout the world. Disposal of biosolids and secondary-treated effluent from wastewater treatment plants either on agricultural land or on non-agricultural areas (golf course and forested areas) are being actively pursued as a mode of reusing the wastes. Excessive loading of these wastes often leads to serious pollution problems. More recently, high molecular weight polymers have been used in agriculture and construction sites for erosion control. A 15 to 50 percent increase in infiltration has been observed at many locations. An enhanced infiltration in soils treated with wastes that contain pathogenic organisms may lead to a faster movement of microorganisms thus increasing the potential for contamination of ground water. However, no literature is available on the movement of pathogenic organisms such as viruses and bacteria as well as chemicals when they come in contact with soils treated with polymers.

The anionic polymers have a net negative charge (negative charged sites exceed in number than the positive charged sites). The viruses are negatively charged colloidal particles. Most surficial soils are negatively charged. Further, the polymers are observed to have strong sorption potential for soils. Research is needed to elucidate mechanisms that contribute to the movement of viruses, bacteria, and other chemicals in polymer treated soils. It is time consuming and relatively expensive to conduct viral assays unless underlying problems with methods are worked out. In the first phase of this project, it was proposed to conduct mobility studies with chemicals and fluorescent microspheres in the first year. A study of the mobility of bacteria *E. coli* in a limited number of tests was proposed in the first year. This proposal (year 2 of the full study) is aimed at studying the transport of FRNA coliphage, two types of bacteria (rod and cocci shaped) and phage sorption and dieaway rates in systems treated with polymers. Refinements to the mathematical model will be carried out once data become available.

Dr. Ray's specialty lies in environmental hydrology of surface and subsurface environments with emphasis on modeling. He also has a strong background in contaminant transport processes including those for viruses. Dr. Fujioka's research is focused on environmental microbiology, virology, and drinking water quality issues. Ms. Yoneyama specializes in environmental microbiology with strong analytical background in virology. This collaboration will not only lead to the assessment of the problem, but it will provide the PIs with opportunity for developing management options for reducing ground-water pollution and for the refinement and validation of a virus transport model.

The proposed research will contribute to the training of a M.S.-level student in environmental microbiology. For the phase 1 of this study, we were successful in recruiting a graduating senior (Mr. T.P. Wong) from the Civil Engineering program for the M.S. program using this research for his thesis. This project gives a unique combination of hydrology and environmental microbiology and prepares the students very well for the job market. This renewal application involves conducting detailed assessment of virus (FRNA coliphage) transport, sorption, and dieaway rates. This will help the graduate student (Mr. Wong) complete his degree. The data will help us prepare additional proposals to agencies such as the U.S. Department of Agriculture, American Water Works Association Research Foundation, and the local agencies.

Research Objectives

The first-year objectives of this research are:

(1) to define the best polymer, dose, and electrolyte that will cause a reduction in soil loss and possible increase in infiltration,

(2) to study the breakthrough of sorbing and non-sorbing chemicals, bacteria sized fluorescent microspheres, and spores through soil columns that are treated with polymers and control,

(3) to examine the breakthrough of the same parameters when the polymers are applied at the soil surface, and

(4) to conduct two breakthrough experiments for bacterium *E.coli* on two Hawaiian soils that were selected from task (a) using secondary treated wastewater.

The second-year objectives of this research are:

(5) to conduct comprehensive breakthrough experiments for bacterium *E. coli* on selected Hawaiian soils that showed enhanced infiltration characteristics with the treatment of anionic polymers.

(6) to conduct experiments on bacterial virus (FRNA coliphage) transport in soil columns treated with these polymers, and

(7) to conduct limited experiments to determine the sorption and dieaway rates of FRNA coliphase under Hawaii conditions and use the data in virus transport modeling.

Methods and Procedures

Both packed and undisturbed columns will be used for this study. Much of the agricultural land, where manure or biosolids are applied, are typically tilled before and after application. Packed columns will be used since the majority of agricultural land in Hawaii is plowed prior to planting. Undisturbed columns have utility for this research from the point of view of preserving the pore structure and they will be collected from a field site preferably where manure has been applied.

Soils and Chemicals: Several tropical soils, (Oxisols, Vertisols, Mollisols, and Aridisols) will be selected for this study. These soils present a degree of contrast for their structure and mineralogy and will be used to conduct a screening study to evaluate the effect of the polymers. Once the preliminary results are obtained, two soils showing the best promise for erosion control and infiltration enhancement will be further studied. For undisturbed columns, a site will be identified where manure, or sludge, or wastewater would have been applied in the recent past. The bulk density and moisture content will be measured *in situ*. From each site, two undisturbed cores (10-cm diameter and 20-cm long) will be obtained. A quantity of soil from each site will be brought for making the packed columns and other chemical analyses. Many of these soils in Hawaii are used for agricultural purposes. Gavenda et al. (1996) provide a detailed physical and chemical characteristics of three Oahu Oxisols. We have obtained several polymers with low to very high charge density from Cytec Industries of Stamford, CT. The low charge density anionic polymer has a very small percentage of amide sites that are substituted with hydroxyl ions. Two cationic polymer of moderate and high molecular weight have been used for comparison. The screening process is nearly over and the summary results are presented in the section "Preliminary Results". Traditionally, anionic high-molecular weight polymers have been used for soil erosion control and they have low aquatic toxicity (R. Sojka, Northwest Irrigation and Soils Research Lab., personal communications, March 1998).

Preparation of Soil Columns: The undisturbed cores from each of the soils will be packed in custom built columns (10-cm ID by 20-cm long) with filters and end plates to act as flow cells. The edges of the columns will be sealed with wax to prevent seepage along the walls. The packed columns will be prepared from air dried soils passing through a 2.0

mm sieve and the column dimension will be the same. The soils will be packed in columns to original bulk density. The columns will be wetted from the bottom to expel the trapped air. Tap water will be used since it is identical to irrigation water in Hawaii. Most drinking water supplies in Oahu are derived from protected watershed with minimal pollution. As a result, chlorination is not practiced unless contamination is discovered.

Apparatus: A column leaching apparatus, made by Soil Measurement Systems of Tucson, Arizona will be used for leaching studies under unsaturated conditions. For saturated experiments, the vacuum system will be disconnected and a higher quantity of water will be applied to maintain saturation or slight ponding. For microbiological analyses, filtration apparatus, centrifuge, incubator, and shakers will be used and all these are available within the laboratory. Bromide analysis will be performed using an ion chromatograph and a gas chromatograph is available for trace chemical analysis. A fluorometer is also available.

Microspheres: Bacteria to protozoa sized particles are available from a number of commercial vendors [e.g., Bangs Laboratories (often marketed under Seragen Diagnostics and Seradyne Particle Technology) and DYNAL (for beads), Polysciences, Inc. (for microspheres)] for research, diagnostics, pharmaceutical, industrial and many other applications. These particles come in various surface charges and can have fluorescent characteristics for easy and quick determination while conducting column breakthrough experiments. Both surface charge and size effects can be studied using the microspheres. The particle densities vary based upon the material of construction. Most carboxylated latex microspheres have a particle density ranging from 1.05 to 1.06 g/mL. The manufacturer specifies the surface charge (meq/g) of these particles. We have selected page and bacteria size carboxylated fluorescent microspheres from Polysciences, Inc. based upon our discussion with researchers who had success in using them in the field (e.g., R. Harvey, U.S. Geological Survey, Denver, CO., 1999)

Screening of Soils and Polymers: A laboratory scale rainfall simulator, available in the Department of Agronomy and Soil Science is being used for screening studies for soils and polymers. Soils will be packed in a soil bin which will be placed on a fixed slope and treated with a given polymer first in dry form and then in solution form at high concentrations. The polymer will be allowed to dry for 30 minutes prior to rainfall. Four polymer doses will be used (5, 10, 15, and 25 kg/ha). Earlier investigation by the PI (see Mitchell et al., 1996) indicated that under field settings, 20 to 40 kg/ha was adequate for to show erosion reduction under Illinois conditions. When needed, gypsum will be applied as an electrolyte to see the effect of adding divalent ions (Ca^{++}) on the effectiveness of PAM. The tap water in Hawaii is naturally low in electrolytes because of its basaltic origin. The amount of soil loss and percolation from each run will be examined for a rainfall intensity of 6.5 cm/hr imposed over a period of 60 minutes. In addition, a small number settling tests will be performed for two polymer doses on these soils to show the flocculation potential of these soils. From this study, two soils and one polymer showing the best results will be selected for flow and transport studies using soil columns.

Flow and Transport Studies: First, the rate of flow of water in undisturbed and packed columns for the two selected soils will be studied under both saturated and unsaturated conditions. Both the retention (pressure versus water content) and unsaturated hydraulic conductivities will be estimated with the leaching apparatus using an upflow technique similar to Toorman et al. (1992). Saturated hydraulic conductivity will be measured by the standard falling head method (Klute and Dirksen, 1986). Once these basic properties are estimated, the flow rate of water under saturated and unsaturated conditions will be determined after adding the polymer. The polymer dose in many erosion studies (see Lentz and Sojka, 1996) has been limited to 10 mg/L in irrigation water and that will be the dose for saturated columns. A comparable dose, best obtained from the screening studies will be used for surface application at high concentrations. If needed, gypsum will be applied along with the polymer. This will provide an indication of the rate of increase in infiltration and water conduction through saturated and unsaturated columns, that are undisturbed as well as, repacked.

In each of the above two sets of experiments, the column effluent will be collected at regular intervals and analyzed for selected anions and cations, pH, turbidity, dissolved organic carbon, and the residual *E. coli* (in the last few experiments). In the subsequent experiments, the water seeded with fluorescent microspheres, chemical tracer (chloride and lithium), the spores of bacterium *Bacillus subtilis,* and *E.coli* will be applied to the soil (flooding for saturated columns and by the syringe pump of the column leaching apparatus for unsaturated columns) and the breakthrough effluent will be analyzed for these parameters. Although the natural soil contains spores or *E. coli* the seed water concentration will outnumber the native population. However, for accuracy, the spores or *E. coli* in effluent will be initially measured prior to adding the seeded water. The chemical parameters can easily be analyzed using a combination of ion chromatography and other wet chemistry methods. The fluorescent microspheres will be counted in a given volume of column effluent using a fluorometer.

Bacteria and Viruses: We will study the movement of bacterial spores (*Bacillus subtilis*), natural spores, and *Escherichia coli* in the beginning. A prototype stain of *E. coli* (ATTC 25922) as well as a natural soil *E. coli* isolate with high resistance to the antibiotic tetracycline will be used for our study. Use of the antibiotic resistant *E. coli* will enable us to be sure that we are always measuring the same population of *E. coli* and will enhance the specific recovery of this *E. coli*. Methods for the specific enumeration of *E. coli* from water and soil are available (AWWA, 1995). Human enteric viruses are the pathogens of contaminant in wastewater, which will most likely contaminate groundwater. However, since the methods for the recovery and enumeration of human enteric viruses from soil and water are expensive, slow and inefficient, it is not feasible to analyze directly for human enteric viruses. An excellent surrogate for human enteric viruses is a virus of *E. coli* called FRNA coliphages because this group of coliphages has the same structure and genetic component as human enteric viruses. They are naturally present in raw sewage at levels approximately 1000 times that of human enteric viruses and the method for their recovery and enumeration is relatively easier and specific. Because of the constraints on time and funding, only a limited number of studies will be conducted using viable *E. coli* (and the remaining will be conducted using fluorescent microspheres) in the first phase. The assay for FRNA viruses will be conducted during the second year of this study. For controls, the prototype FRNA coliphage (MS2) will be used. This coliphage can be easily grown to high culture in the special *E. coli* strain HS (pFamp) R and can be diluted in water, buffer or sewage to be applied to experimental soil samples. The *E. coli* HS (pFamp) p strain will also be used to specifically enumerate the populations of FRNA coliphages from soil samples to determine their expected survival and transport through soil with and without treatment with polymer. All virus work and most bacterial analyses will be conducted in the second year.

Microbiological Analyses of Effluent: The effluent will be diluted or concentrated depending upon the concentration of *E. coli* and phage. If the concentrations are high enough, the samples will be serially diluted and a given amount of the sample will be directly enumerated on the culture media or host cells. The inoculated media will be incubated and the colonies will be counted. The reported units will be the number of colony forming units (CFU) per mL of water. However, if the samples are low in organism count, it will be necessary to concentrate the samples. For *E. coli* analysis, a given amount of water will be filtered through a 0.45 _m cellulose acetate membrane. The filter will be placed on an agar medium and incubated. For spore analysis, the effluent water samples will be heated to 60 ^{0}C and kept at that temperature for 15 minutes to destroy all vegetative cells. The spore will survive these environmental conditions and will be cultured on a nutrient agar such as tripticase soy agar following Standard Methods (1995) procedures. The *E. coli* analytical procedures will remain same during the second year. For phage, an electropositive membrane (0.20 _m) will be used for filtering and the phage on the membrane can be extracted with several media (beef extract, NaCl, glycene-NaoH). The extracted phage will be assayed on a lawn of host *E. coli* cells. The phage will be reported in number of plaque forming units (PFU) for mL of water.

Microbial Sorption and Dieaway Studies: Sorption of bacteria and FRNA coliphage to the two Oahu soils will be conducted in laboratory conditions in year 2 using a simple batch sorption technique. A measured (small) quantity of soil will be added to the seeded solution of the FRNA phage that is near neutral pH and well buffered and mixed thoroughly for several days. Samples will be removed at various times and the FRNA phage will be recovered and enumerated from the solution and the soil. The same technique will be followed for the solution that contains 5, 10, and 25 mg/L of polymer solution. In the past, recovery of phage has been found to be variable among samples. We will take multiple samples for reducing the variability. A similar study will be conducted for *E. coli*. We will also perform a dieaway experiment on the FRNA phage by subjecting the mixture (soil and phage after equilibrium sorption) to various temperatures and assaying for viable phage and quantifying those. These information will be useful for modeling.

Besides the above batch method, we will attempt to estimate the sorption distribution coefficient from a column leaching experiment at a controlled temperature and measuring the breakthrough concentration as a function of time. We will establish a steady-state leaching condition by applying a conservative tracer followed by the seeded FRNA solution at a rate lower than the saturated hydraulic conductivity of the column. Once the flow rate and hydrodynamic dispersion coefficients are estimated from tracer breakthrough data, we will use those information and the dieaway rate at that temperature to estimate the sorption distribution coefficient of the FRNA phage using an inverse technique (e.g., Toride et al., 1995).

Soil Sampling and Analyses: After the experiments are over with bacteria and the phages, we will open the soil columns and collect soil samples at three depths (near surface, middle, and bottom). Sorbed microspheres and bacteria will be extracted from a given amount of sample by elution and the samples will be analyzed by the traditional culture method. If the results show deeper migration of bacteria, spores, and phage, we will conduct both culture and possibly PCR work (contractually) for a small number of samples during this proposed second year.

Enhancement of Mathematical Modeling Capability (2^{nd} year): The water flux and sorption behavior of FRNA coliphage and bacteria in soils that are treated with polymers can different than those without polymer treatment. Ray and Espinoza (1996) have developed a virus transport model that accounts for the movement of viruses in the vadose zone and the flux from the vadose zone is used as the source for a 2-dimensional saturated zone. The vadose zone flow and transport model uses the Galerkin finite element method, whereas the 2-D saturated zone model uses finite elements for flow and the Random-Walk technique for transport with linear kinetic sorption. The vadose-zone model will be slightly modified to include a colloid filtration component (see Hydrogeologic, Inc., 1994). The two-dimensional saturated zone model will be further evaluated for the effect of one or more pumping wells and linear kinetic sorption using the Random-Walk method. Using the measured and available parameters, the estimated concentrations for a given source with and without polymer treatment will be simulated. In addition, the model CANVAS (Hydrogeologic, Inc., 1994) that was developed for the USEPA for the proposed Ground Water Rule will be compared. More effort will be spent on modeling issues for scenarios relating to source-water protection for drinking water supplies.

The modeling issues are quite important for evaluating the effect of different management scenarios. The results of 2^{nd} year effort will help us in getting model parameters for calibration and testing purposes. The payoff of these analytical efforts could be reflected in the accuracy of this model.

Health Concerns for the Graduate Student and PIs: The chemical and fecal organisms proposed for use in this study do not pose serious health impacts unless the researcher ingests them. This is unlikely to happen since the laboratory QA/QC protocols require the student to use proper protective measures. Further, no food and drinks are stored in the microbiology laboratory. As an additional safety measure, we will use a nonpathogenic strain of *E. coli* in stead of the strain, which can be found in septic tanks or sewage. Details of the non-pathogenic nature of the *E. coli* and the phage are presented earlier.

Research Facilities

The Environmental Engineering Laboratory in the Department of Civil Engineering is equipped to conduct analyses of bacteria and viruses. Epifluorescence microscopy, freezers, refrigerators, centrifuges, ion chromatographs, a fluorometer, and large autoclave facilities are available. In addition, walk-in temperature controlled rooms are available in both the laboratories. Computing facilities are adequate for data analysis. Ms. Yoneyama is the overall manager of the laboratory. A seed money grant from the University of Hawaii has enabled Dr. Ray to purchase a column leaching apparatus that is being used in this project.

Related Research

Numerous authors have studied the survival and mobility of pathogenic bacteria and viruses in soils and ground water in the past 20 to 25 years since land treatment of wastewater became popular in late seventies. The research on the use of polymers for soil erosion control is relatively new (6 to 7 years). While the results on erosion reduction has been astounding, the knowledge on the impact of polymers on enhancing infiltration is limited. Further, literature on the impact of polymers on the leaching of chemicals and microorganisms does not exist.

Use of polymers in agricultural settings has primarily been in furrow irrigation (Lentz and Sojka, 1996) and now 600,000 acres in the Unites States are treated with polymers for erosion control. More than 95 percent reduction in soil loss in the tail end of the furrows has been observed (Lentz et al., 1992; Lentz and Sojka, 1994). With the use of polymers, accompanying increases in water infiltration beneath the furrows ranging from 15 to 50 percent have been observed (Sojka and Lentz, 1994; Lentz and Sojka, 1994; Trout et al., 1995; Sojka et al., 1996). Mitchell (1986) observed a 30 to 57 percent rise in infiltration during furrow advance. Figure 1 shows increases in infiltration observed by several authors. Roa (1996) examined the impact of several polymers, soil amendments (electrolyte source) and pH adjustment on the flow of water through saturated Plano silt loam soils under laboratory conditions. In the first two hours, he observed a significant enhancement for flow for columns that were treated with polymers. The addition of electrolytes to the polymer-amended water and adjustment (lowering) of pH further improved the flow rate of water. Letey (1996) found that polymer viscosity can reduce flow in silica sand (where no fine particles are present).

Several bacteria, protozoa, and viruses can be found in pathogenic animal wastes, wastewater, and biosolids. *E. coli* and *Salmonella sp.* are common pathogenic bacteria in animal feces. Protozoa such as cryptosporidium and giardia are derived from several domesticated and undomesticated animals. The analytical techniques for many of these protozoa are still complex and expensive. The culture and analysis of enteric viruses are equally expensive and time consuming for monitoring studies (Sobsey and Handzel, 1994). Many virological studies in the past 20 years have lead to the use of bacterophages (phages) as acceptable indicators of water pollution by animal enteric viruses (Gerba, 1987; Goyal 1983; Hilton and Stotzky, 1973; Kott et al., 1974; Scarpino, 1975; Snowden and Kliver, 1989). In addition to these and other published studies, we are in communication with over a dozen microbiologists from around the country for the proposed Ground-Water Rule (GWR) of the U.S. Environmental Protection Agency. The use of phage as virus indicators is widely accepted.

Phages are viruses that infect bacteria as their host cell; those that infect E. coli (coliform bacteria) are termed coliphages. As members of the virus kingdom, they share similar characteristics with animal viruses. For example, Bitton (1982) showed that a number of physical and chemical characteristics phage f2 (a F-specific RNA coliphage, FRNA) closely resemble those of poliovirus, a human enteric virus; both are small, icosahedral, single stranded RNA viruses. FRNA coliphages are found in high concentrations in fecal samples of pigs, sheep, calves, and chicken (Havelaar et al., 1986). Among the advantages of detecting coliphages over enteric viruses are that coliphages are present in abundance in polluted waters often exceeding the concentration of enteric viruses present (1:1 to 1000:1); can be isolated and enumerated by relatively simple methods and shorter time intervals than required for assaying enteric viruses; and may be more resistant to inactivation than enteric viruses (Kott, 1974; 1981). In addition, coliphages can be cultured and assayed in the laboratory at a cost, which is only a fraction of that for the assay of enteric viruses, by the PCR (gene probe) techniques.

Sorption and desorption of viruses to soils and other solids are governed by electrostatic double-layer interactions, van der Waals forces (Gerba, 1984), hydrogen bonding, covalent-ionic interactions, and hydrophobic effects (Bales et al., 1991). Because of its electrostatic nature, adsorption can be partially reversible. Thus a quantitative description of adsorption and desorption is essential for modeling. Physical and chemical properties of the porous medium and water (soil texture, water velocity, water content (unsaturated soil), pH, ionic strength, organic matter content etc.) affect sorption and desorption. In addition, the surface properties of the virus affect sorption. Finer particles are good adsorbents for viruses. Lance and Gerba (1984) also found that adsorption of viruses was higher under unsaturated conditions. One of the explanations was that under unsaturated conditions, the thin water films bring viruses close to soil particles. Soil particles carry a net negative charge under natural conditions, especially under temperate regions. The isoelectric point (IP) for many enteric viruses is below 7, thus at neutral pH, most viruses are negatively charged. Adsorption will be enhanced if the repulsion between the negatively charged virus and soil particles is reduced. More saline water (e.g., high in ionic strength) may exert a net positive electrical potential near neutral pH. If the viruses have a net negative charge under these conditions, it will lead to greater adsorption of viruses (Gerba, 1984). Presence of organic matter interferes with the sorption behavior of viruses. Not only they compete with viruses for sorption sites; they also cause elution of sorbed viruses. Soils with high organic carbon (e.g., muck, peat etc.) may be poor adsorbers of viruses and such soils may not be suitable for wastewater disposal (Yates and Yates, 1988).

Three common methods are used for getting adsorption parameters: two based upon equilibrium sorption (Langmuir and Freundlich isotherms) and one using the kinetic adsorption (Bales et al., 1991; Corapcioglu and Haridas, 1985; Gerba 1984; Moore et al., 1981). In the Freundlich isotherm, the exponent is shown to vary from 0.87 to 1.24 (Vilker and Burge, 1980). If the exponent is 1, the isotherm becomes linear. The linear isotherm is commonly used in many modeling practices for solutes (Fetter, 1993) and viruses (Hydrogeologic, Inc., 1994). Although equilibrium sorption experiments are easy to conduct, kinetic models are more appropriate to simulate field conditions. Harvey and Garabedian (1991) present a simple linear kinetic first-order model, which accounts for forward and reverse adsorption rates. Ray and Espinoza (1996) use the same model and solve it using the Random-Walk technique.

Virus inactivation or dieaway rates are important for the mobility and survival rates of virus under differing environmental conditions. Temperature is considered to be the most important factor affecting the survival of viruses in subsurface (Bitton, 1980; Bales and Li, 1993; Yates and Yates, 1988). Low temperature (Sobsey, 1983) and high soil-water content (Yeager and O'Brien, 1979) are favorable for the survival of viruses in subsurface. Inactivation is enhanced with the drying of soils (Hurst et al., 1980a; Powelson et al., 1990). Viruses behave differently to ambient conditions. For example, polio- and echoviruses live longer in sterile soils than non-sterile soils. Hurst et al. (1980b) reported that survivals of these two virus types are similar in the two soil types near freezing temperatures, however the survival was higher in sterile soils at higher temperatures. Bitton (1980) also suggest that formation of virus aggregates improve the viruses' ability to survive under adverse environmental conditions. The effect of adsorption on virus inactivation is inconclusive.

Often first-order decay rates are used to represent virus inactivation in the subsurface (Corapcioglu and Haridas, 1984; Reddy et al., 1981; Yates and Ouyang, 1992). Yates and Yates (1987) propose a temperature based linear equation to obtain the decay coefficient for first-order models.

Modeling of virus movement in subsurface has been proposed by a number of researchers. Most are one-dimensional models. Available one-dimensional models include VIRALT (Hydrogeologic, Inc., 1994) and VIROTRANS (Tim and Mustaghimi, 1991). Similarly the two-dimensional models include CANVAS (Hydrogeologic, Inc., 1994) and that by Ray and Espinoza (1996). As expected, the models vary in terms of their sophistication and numerical solution methods. The key problems using any model is the soundness of model parameters. It has been hotly contested in recent years about the possible use of virus transport models for setting setback distances of contaminant sources for drinking water wells. Because of parameter uncertainty, the utility of these models, at this time, is less important. However, one should make an effort to check the validity of these models when realistic data are available.

Microspheres have been used in transport research in the recent years. Harvey et al. (1989) studied the microspheres and indigenous bacteria during natural and forced gradient tracer tests at the Cape Cod experiment site. The aquifer is composed of fine to medium sand. Microspheres used for the study ranged in size from 0.23 to 1.35 _m in diameter made of carboxylated latex. The surface charge for these microspheres varied excepting the microspheres of 0.6-_m diameter that were uncharged (made of latex). The advantage of using microspheres was the ease of analysis for a large number of samples. Harvey et al. (1995) also used protozoa-sized microspheres (2-_m diameter) that were carboxylated and surface active and they simulated well the movement of protozoa at the same site compared to the bacterial study cited above.

Preliminary Results

PI Ray has been examining the effect of high molecular weight polymers (PAMs) on the reduction of soil erodibility and runoff for selected Hawaiian soils. After various trials on dose and application methods, at present most soils seem to produce lower soil loss under polymer treatment with the exception of Vertisols. Vertisols are expansive soils with a significant amount of montmorilonitic clay minerals. Once the simulated rainfall is applied on the dry soil surface, the soil completely seals without producing any infiltration. However, it has been observed that the addition of PAM to Vertisol surface reduced the soil loss compared to controls, especially, the splash component was quite low.

The results are here are grouped into three areas: (a) soil loss reduction and infiltration enhancement, (2) particle flocculation, and (3) aggregate stability following PAM application.

A laboratory scale rainfall simulator, available in the Department of Agronomy and Soil Science was used for this study (Figure 1). It is a drip-type simulator that covers an area of 76 cm by 76 cm and has 841 drop forming needles each forming 3.2-mm drops. The chamber that holds the drop making needles can be raised to a maximum height of 2.3 m within the laboratory. At present, it is operating at a height of 2.3 m and the kinetic energy of the simulated rainfall is 16 J/m^2/mm of rain. Natural rainfall intensity for a 60-mm/h rain is 27 J/m^2/mm. Thus the laboratory simulator has the capacity to produce 60% of the energy of the natural rainstorms. The rainfall intensity was controlled by a pressure regulator and was monitored by a pressure gage. Two oscillating fans were used to prevent raindrops from falling repeatedly at the same place. The simulated storms were high intensity (5-8 cm/hr) storms of duration 60 minutes.

oil trays measuring 30_30_12 cm were filled with 3-cm of coarse sand at the bottom. A metallic screen was placed on the top of this sand layer to prevent the top 9-cm of soil to mix with the sand. A drainage collection system is located at the bottom of each soil tray. The bulk density of the packed soil was similar to that found in the field. All soil trays were placed inside an open-ended-wax coated wooden box. The box received the rainfall from the simulator and the runoff and splash from the box was collected through a metal trough attached to the front of the box.

In the initial screening, several polymers were obtained from Cytech Corporation of Stamford, CT. Table 1 provides a summary of some of these polymers tested on the four soils studied. Superfloc A836 is the most commonly used PAM in agricultural settings. PAM C-492 is a cationic polymer, however, cationic polymers are rarely sued in natural settings due to their potential aquatic toxicity. In the initial process, PAM was applied in dry form to the soil surface. Since the quantity of application was low compared to surface area, PAM was sprinkled over the soil surface using a salt shaker. Once the soil trays were subjected to simulated rainfall, the polymer dissolved and began to work on the soils. Because of longer dissolution time, each simulation run was 60 minutes after the first production of surface runoff. Subsequently, the application method was changed since the uniformity of application was a problem and dissolution was expected to cause uneven distribution of PAM on the soil surface. For this reason, a high-pressure sprayer was used to apply PAM at 450 mg/L directly at the soil surface at the needed doses. In each run, the PAM applied tray is subjected to rainfall under the given conditions. Once the needed data are collected, the soil trays are stored for 24 hours in the laboratory in a plastic bag. The same rainfall simulation is conducted on the second day (when the soil surface is wet) without any further addition of polymers.

Figure 2 shows the impact of PAM treatment (in the form of 450 mg/L solution to soil surface) on infiltration in a Wahiawa Oxisol. As can be observed from this figure, most of the improvement in infiltration occurred at an application rate of 10 kg/ha with minimal improvement at higher doses and nearly 98% of applied rainfall was converted to percocolate.

Table 2 provides summary of sediment concentration in Hoolehua and Wahiawa soils function of dose for three simulations (both dry and wet runs). The same table also provides runoff coefficient data the same two soils and the Hilo soil (an Andisol). As can be observed from this table, sediment concentration in runoff water reduced with polymer dose. The runoff coefficients were significantly low for Hoolehua and Wahiawa. The Hilo soil behaved differently in the second and third runs. More investigation is being carried out on this soil taking into account the physicochemical properties. Given the fact that most Hilo soils are wet year round. Soil preparation might have contributed to some of these discrepancies.

Particle flocculation is a relative measure of settling rate of fine particles in water. High turbidity water causes a reduction in infiltration by blocking soil pores. Once the particles are settled with PAM, the clear water can pass through the soil easily. The residual PAM can keep the fine particles around the pores in flocculated state, thus preventing resuspension. Figure 3 shows particles settling for three soils as a function of polymer dose.

Aggregate stability is a measure of the relative resistance of particles to breakdown due to environmental factors such as rainfall impact, water flow, and friction. Aggregate stability for the Vertisol soil several polymers and doses are presented in Figure 4. As can be observed, the Superfloc A-836 appeared to have the best effect in retaining the aggregates in the upper sieves.

At present, the column leaching apparatus (Figure 5) is functional and bromide leaching with and without polymer addition for sand and soil columns is being carried out. Bromide analysis is being carried out using an ion chromatograph. After this, experiments with microspheres will be carried out.

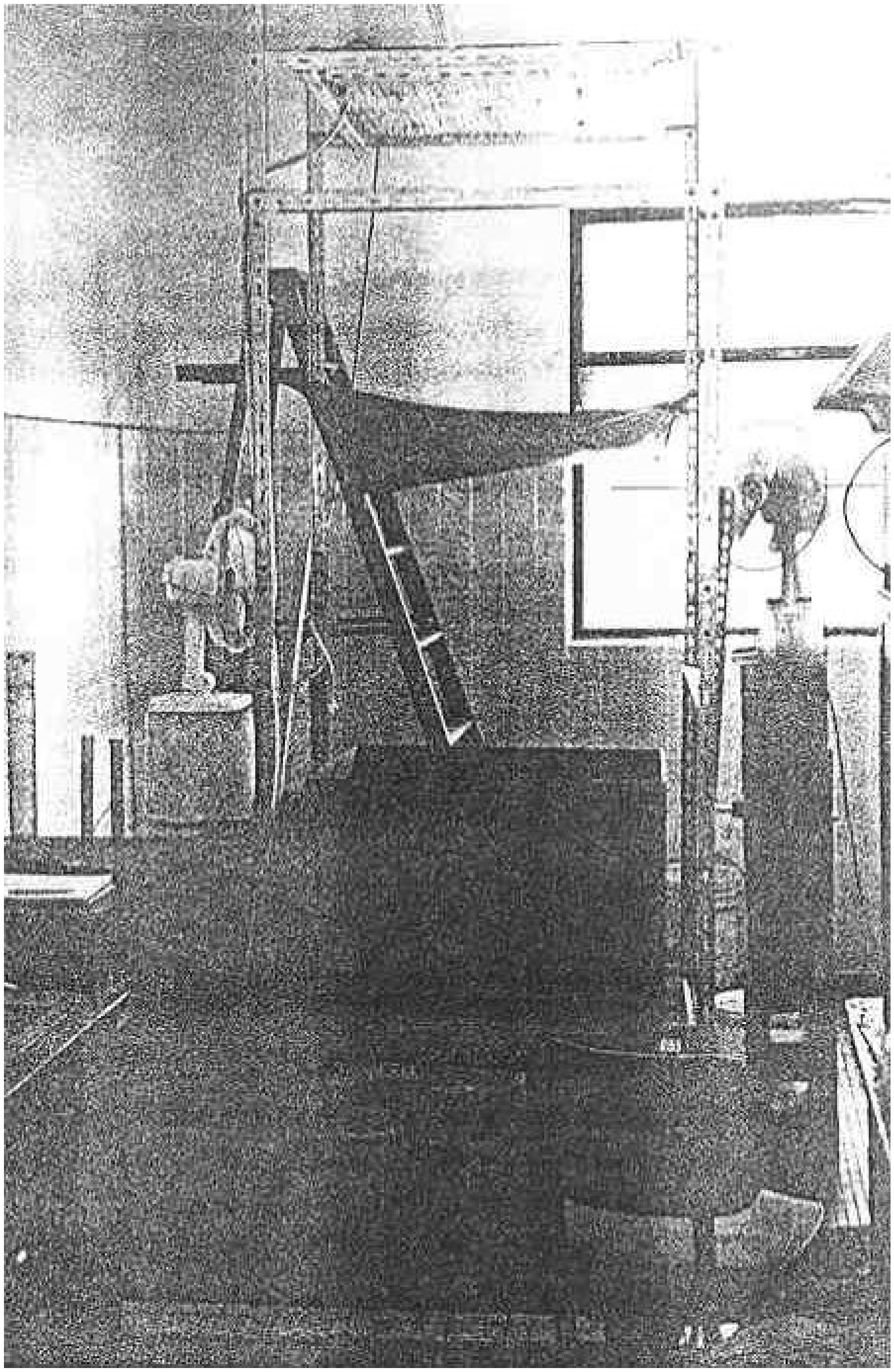


Figure 1. Rainfall simulator setup.

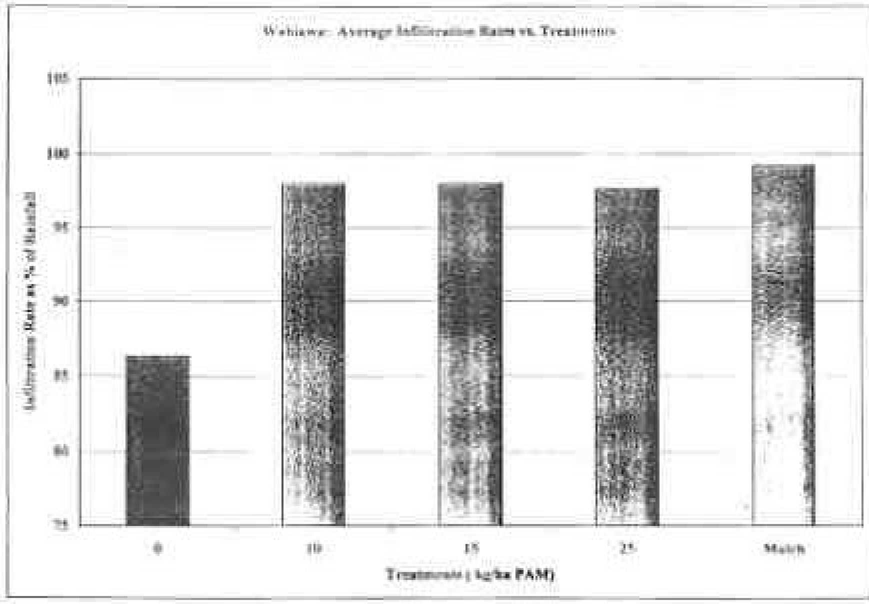


Figure 2. Infiltration enhancement in Wahiawa Oxisol as a function polymer dose.

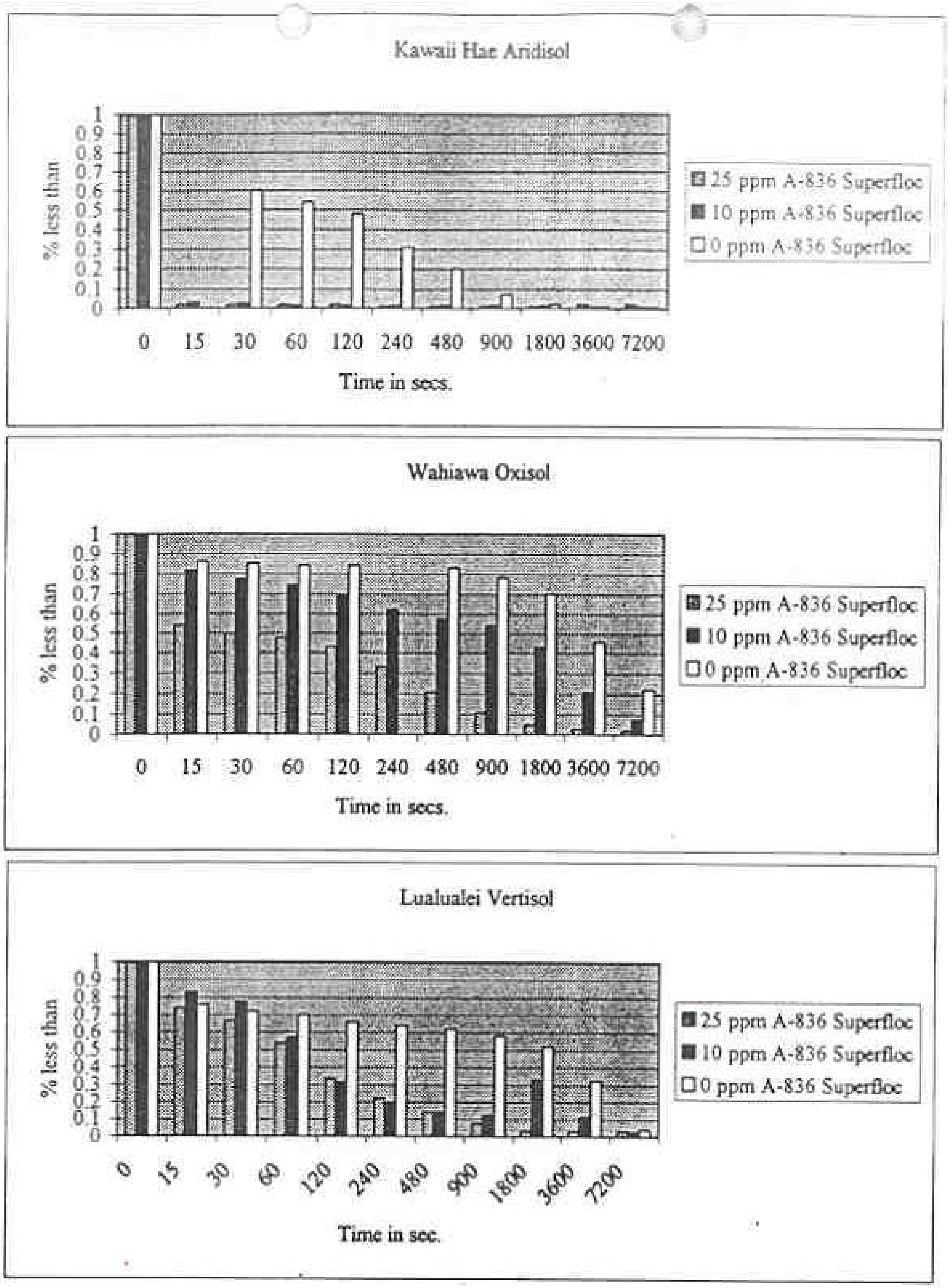
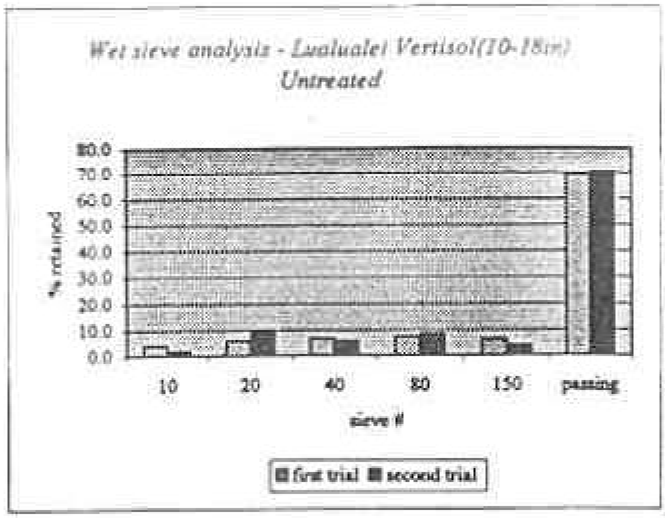
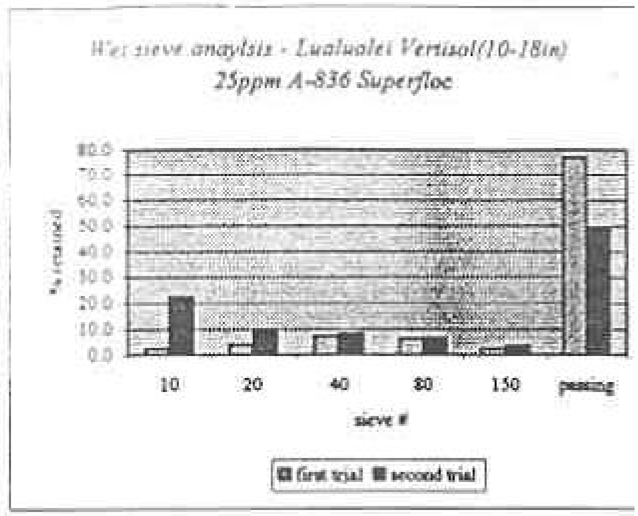


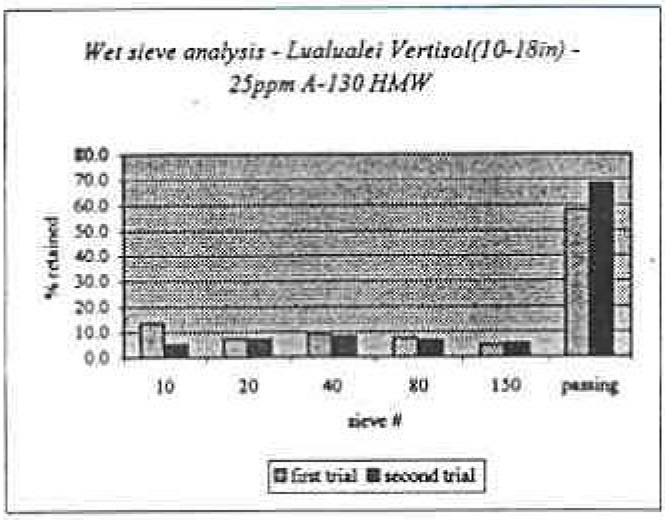
Figure 3. Particle settling rates of three tropical soils as affected by polymer dose



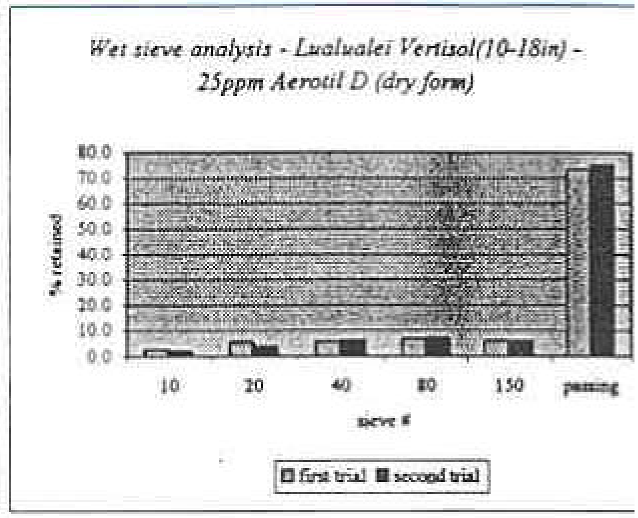
Wet sieve analysis - Lualualei Vertisol(10-18in)
Untreated
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% retained
10
20
40
80
150
passing
sieve #
first trial
second trial



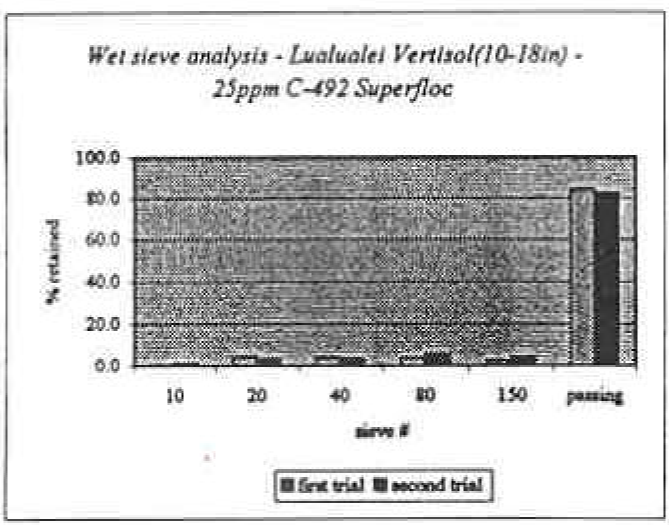
Wet sieve anaylsis - Lualualei Vertisol(10-18in)
25ppm A-836 Superfloc
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% retained
10
20
40
80
150
passing
sieve #
first trial
second trial



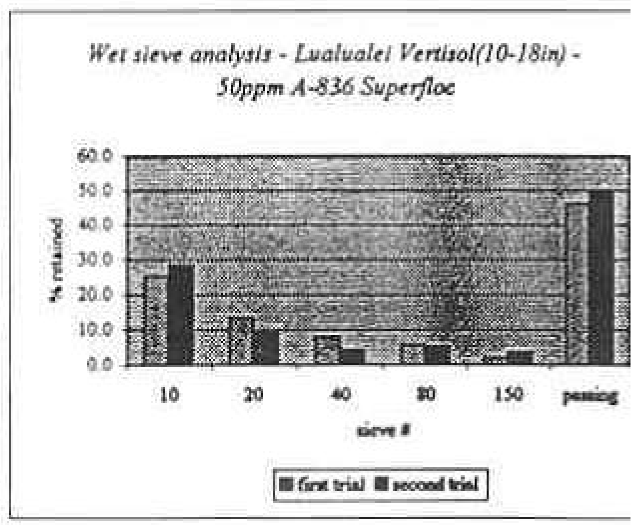
Wet sieve analysis - Lualualei Vertisol(10-18in) -
25ppm A-130 HMW
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% retained
10
20
40
80
150
passing
sieve #
first trial
second trial



Wet sieve analysis - Lualualei Vertisol(10-18in) -
25ppm Aerotil D (dry form)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% retained
10
20
40
80
150
passing
sieve #
first trial
second trial



Wet sieve analysis - Lualualei Vertisol(10-18in) -
25ppm C-492 Superfloc
100.0
80.0
60.0
40.0
20.0
0.0
% retained
10
20
40
80
150
passing
sieve #
first trial
second trial



Wet sieve analysis - Lualualei Vertisol(10-18in) -
50ppm A-836 Superfloc
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% retained
10
20
40
80
150
passing
sieve #
first trial
second trial

Figure 4. Aggregate stability for Lualualei Vertisol with varying polymers and doses

Figure 5. Set up for column leaching apparatus(not provided)

Table 1. Properties of selected polymers received as samples from Cytech Industries.

Product	Approximate molecular weight (10^6 grams/mole)	% charge
Supefloc A836	15-16	18 (-)
Superfloc A130*	16-18	34 (-)
Aerotil-liquid	0.2	75(-)
Aerotil-dry	0.2	75(-)
Pristine	15-16	30(-)
Wp6001	0.2	8(-)
Cyanamer N-100L	**0.015**	**nonionic**
C-492	5	8(+)

*New name for Superfloc A866

Table 2. Sediment concentration and runoff coefficient for three soils

Soil type with dose	Run 1	Run 2	Run 3
Sediment Conc. (g/L)			
Hoolehua (kg/ha)			
0	1.38	1.34	1.24
10	1.37	1.66	1.05
15	0.72	1.16	0.71
25	0.85	0.18	0.33
Mulch	0.21	0.07	N.A.
Wahiawa (kg/ha)			
0	1.50	1.42	1.39
10	0.68	0.74	0.77
15	0.72	0.79	0.86
25	0.20	0.90	1.80
Mulch	1.61	0.96	0.00
Runoff/Rainfall			
Hoolehua (kg/ha)			
0	0.16	0.24	0.14
10	0.07	0.08	0.19
15	0.12	0.19	0.11
25	0.13	0.07	0.07
Wahiawa (kg/ha)			
0	0.18		